Epitope Tagging Basic Laboratory Methods

Exhibit A

last update April 26, 2000

Chapter 1: Introduction to Epitope Tagging

Chapter 2: Molecular Tagging

Section 1B
About Epitope Tagging

What is epitope tagging?

An **epitope** (also called an antigenic determinant) is any structure or sequence that is recognized by an antibody. A single large molecule such as a protein may have many epitopes (Figure 1B.1).

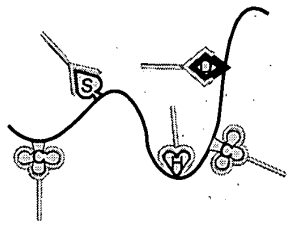

Figure 1B.1: Epitopes. Epitopes C, S, H, and D are structures on a single large molecule that are each recognized by a different antibody. An epitope (such as C) can be recognized by the same antibody even when it occurs at different locations on the same molecule or on different molecules.

For many years, laboratories have raised antibodies to epitopes on naturally occurring proteins (Evan *et al.*, 1985; Wilson *et al.*, 1984; Kreis, 1986) to track the function, movement, and modification of the protein *in vivo* and *in vitro*. Epitope-specific antibodies have also been used to purify the protein that contains the epitope (Kreis, 1986).

Most importantly, many experiments have shown that a single epitope may occur on (or be added to) different proteins and still be recognized by the same antibody (Kolodziej and Young, 1991). With this realization, **epitope tagging** (Figure 1B.2) was born.

Many laboratories (for instance, Cravchik and Matus, 1993; Duden *et al.*, 1991; Field *et al.*, 1988) began using recombinant DNA techniques to transfer a small peptide epitope to a protein that did not normally have the epitope. Then, the epitope-tagged protein could be tracked, analyzed and purified with an already existing tag-specific (that is, epitope-specific) antibody.

Note: For more on the applications of the epitope tagging technique, see Table 1B.1 on page 1.4.

Why tag proteins?

Epitope tagging is an attractive, simple technique that can answer important and difficult questions about almost any protein.

For example, imagine that you have discovered an interesting new gene, GEN18, in the organism you are studying. On sequencing GEN18, you find that it contains an open reading frame that could encode a protein, p18. However, you would like to know:

1. Where is p18 located in the cell?
2. What other proteins does p18 interact with?
3. What is the function of p18 and how does it respond to changes in cell conditions?
4. How does p18 move within the cell during its lifetime?
5. What is the subunit structure of p18?

Epitope tagging can help you find answers to all those questions and more (Table 1B.1) about p18. Even if you only know part of p18's DNA coding sequence, you can fuse an

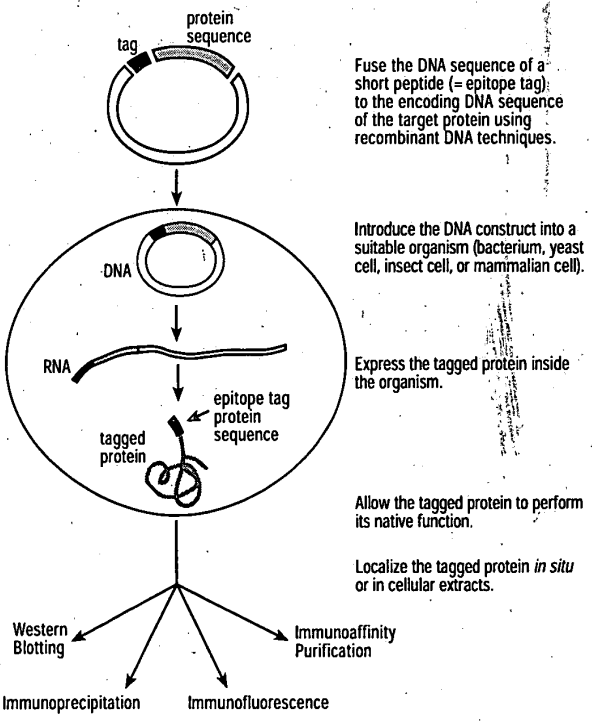

Figure 1B.2: Schematic of epitope tagging technique.

epitope coding sequence to the p18 sequence. The epitope tag will then go everywhere the target protein does, *in vivo* or *in vitro*. You can use a commercially available tag-specific antibody to find and analyze tagged p18 anywhere in the cell. The same antibody can be used to purify and characterize p18.

In addition to its flexibility and wide application (Table 1B.1), epitope tagging offers several advantages over other methods of analyzing and purifying proteins:

- Epitope tagging is much faster than the traditional method of producing a new antibody to every protein studied. The same tag-specific antibody will recognize the epitope tag in many different proteins.
- Epitope tagging is much less costly and labor intensive than setting up and maintaining antibody-producing facilities.
- Adding a small (3–14 amino acid) epitope tag generally does not affect the function of the tagged protein, allowing the study of the tagged protein's role in the cell. This contrasts with the addition of a much larger polypeptide to a target protein to form a fusion protein; the fused polypeptide may alter the function of the target protein.
- Epitope tagging makes it possible to gather information about proteins that would otherwise be too difficult to purify or too similar to other proteins to be distinguished *in vivo*.

Object of research*	How epitope tagging was used
Subcellular localization of tagged proteins	Immunofluorescence analysis of tagged proteins in permeabilized cells
	Ultrastructural analysis of tagged proteins in cells with gold-conjugated tag-specific antibodies and electron microscopy
	Western blot analysis of tagged full-length and truncated proteins in cell membrane subfractions
Determination of protein-protein interactions	Immunoprecipitation of tagged protein from cell extract and gel analysis of precipitate
	Immobilization of tagged protein on Protein A-agarose to study *in vitro* assembly of a multiprotein complex
Functional assay of tagged proteins	Immunoprecipitation of tagged protein from cell extract and activity assay (for example, phosphorylation) of immunoprecipitate
	Western blot detection of tagged protein in cellular extracts under varying conditions (for instance, activation or suppression of a cell function)
Tracking movement of tagged proteins within a cell	Immunoprecipitation of tagged protein from cell extract after pulse-chase labeling of cellular proteins
	Immunofluorescence analysis of tagged proteins in intact cell membranes
	Localization of tagged proteins in cells with gold-conjugated tag-specific antibody and electron microscopy
	Localization of tagged proteins in cells with confocal immunofluorescence microscopy
Characterization of new proteins	Western blot analysis of tagged proteins expressed by transfected cell lines
	Purification of tagged protein from cell extract by affinity chromatography
	Immunoprecipitation of tagged protein from cell extract and gel analysis of subunit structure

*For examples, see references listed under these topics in Section 6C, pages 6.10–6.16, of this manual.

Table 1B.1: Experimental uses for epitope tagging

References

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